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ORIGINAL ARTICLE

Lipocalin-Type Prostaglandin D Synthase and Egg White Cystatin React with IgE Antibodies from Children with Egg Allergy

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ABSTRACT

Background: Ovalbumin, ovomucoid, ovotransferrin, lysozyme, and ovomucin are known to be major allergens found in egg white. Egg white protein is composed of over 30 proteins; many of which have neither been identified nor their allergenicities characterized. This study set out to analyze whether unknown proteins that bind to IgE antibodies in serum from patients with egg allergy exist in egg white.

Methods: Diluted egg white proteins were separated by 2-dimensional (2-D) gel electrophoresis. Immunolabeling was performed on individual patient sera from 19 child patients with egg white allergy and 11 negative control subjects. Spots of egg white proteins that bound to the patients' IgE were identified by mass spectrometry-based proteomics.

Results: Egg white proteins were separated into 63 spots. Twenty-five of the 63 reacted with egg allergy patients' sera, and 10 of the 25 reactive spots showed IgE-reactivity to controls as well. Specific bindings to the IgE from egg allergy patients were found in 15 spots; one of which was confirmed as ovotransferrin. Among the other 14 protein spots, egg white cystatin and lipocalin-type prostaglandin D synthase (L-PGDS) were newly identified proteins that reacted with IgE in patients with egg allergy.

Conclusions: We demonstrated that L-PGDS and cystatin reacted with serum IgE in patients with egg allergy. Our proteomics-based analysis in egg white gives a comprehensive map of proteins bound with IgE and should assist in enabling more accurate diagnoses and recommendations of desensitizing treatments for individual patients.

KEY WORDS

cystatin, food allergy, hen's egg white, LC-MS/MS, lipocalin-type prostaglandin D synthase

ABBREVIATIONS

2-D, two dimensional; MALDI-TOF/MS, matrix assisted laser desorption ionization time of flight mass spectrometry; LC-MS/MS, liquid chromatography/tandem mass spectrometry; L-PGDS, lipocalin-type prostaglandin D synthase.

INTRODUCTION

Hen egg white, which is widely used in processed foods, is one of the most common and serious causes of immediate food allergic reactions in infants and young children.[1,2] This is because of their immature gastrointestinal epithelial membrane barrier that allows more proteins to enter the circulation.[3,4] Egg allergy induces vomiting, diarrhea, abdominal pain, urticaria, angioedema, acute rhinoconjunctivitis, cough, wheezing, and anaphylactic shock. It is well known that egg white protein is composed of over 30 pro-

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Table 1 Clinical characteristics and egg-white RAST scores in 19 patients with egg white allergy

Patient	Age	Sex	Clinical reaction		Total IgE (IU/ml)	Egg white IgE (UA/ml)	RAST egg white
			Reported episode	Food challenge			
SH	2	F	Systemic anaphylaxis, urticaria		34.7	1.6	2
SK	4	M	Angioedema		1347.5	44.1	4
HS	1	F	Urticaria		114.4	5.4	3
AO	1	M	Erythema	Erythema, urticaria	137.6	11	3
RS	1	M	Cough, wheeze		181.8	10.6	3
TE	5	M	Urticaria	Abdominal pain, urticaria, cough	509.9	7.2	3
AK	2	F	Urticaria	Urticaria, diarrhea	326.4	6.73	3
KM	4	F	Urticaria	Urticaria	741	24.2	4
RH	4	M	Urticaria	Urticaria	895.3	11.3	3
KK	2	M	Pruritus		630.4	20.2	4
AS	2	F	Dyspnea, vomiting, urticaria		55.3	0.5	1
YY	1	M	Urticaria, angioedema	Erythema, urticaria	160	3.9	3
MY	1	F	Urticaria		181.4	4	3
KS	0	M	Diarrhea		1497.7	>100	6
MT	6	M	Erythema		550	1.3	2
HM	1	F	Erythema	Erythema	600	15	3
KS	1	M	Erythema	Cough, pruritus	200	2.5	2
SS	3	M	Urticaria	Urticaria	56	1.3	2
RY	0	F	Erythema		28	1.8	2

GI, Gastrointestinal symptoms; resp, respiratory symptoms.

teins.[5] The allergenicities of egg-white proteins, with the exception of ovalbumin, ovomucoid, ovotransferrin, lysozyme, and ovomucin have not been analyzed.[6-8]

Recently, new methods for analysis and characterization of proteins, including 2-D electrophoresis techniques and mass spectrometry, have been developed.[9-11] Liquid chromatography/tandem mass spectrometry (LC-MS/MS), or matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF/MS), coupled with search engines to access the huge amount of data produced by the genome sequencing projects, has fueled the growth of proteomics.[12,13] Since spectra from only a few peptides (or even a single peptide) can be sufficient to identify a protein, new allergens have been reported using these methods.[14-17] It has also been reported that the combined use of MALDI-TOF MS and LC-MS/MS has significantly increased the total number of identified proteins.[18,19]

We sought to characterize any unknown egg white allergens using proteomic approaches. Sixty spots were visualized in egg white on 2-D gel using silver stain. We then analyzed egg white proteins that bound to serum IgE in patients with egg allergy on 2-D immunoblots; two of the spots that bound to IgE were subsequently newly identified by LC-MS/MS and were confirmed to react with IgE by immunolabeling using each purified protein.

METHODS

SUBJECTS

Sera from 19 patients with egg allergy (aged 2.2 ± 1.7 years, total IgE 434.1 ± 424.7 IU/ml), and 11 negative control subjects (4.0 ± 3.7 years) with no clinical history of any allergic symptoms were obtained and stored in aliquots at -80℃ (Table 1). Informed consent for the study was obtained from the parents of all participating subjects before collecting blood, and the protocol was approved by the Ethics Committee of Gifu University School of Medicine. Diagnosis of egg white allergy was based on clinical history and CAP-RAST results to hen's egg white.[20] Food challenges were performed in an open manner for 9 of the 19 patients without severe reactions. Our first challenge dose was 1/40 of boiled egg white, and the dose was doubled every 15 minutes until the patient exhibited distinct symptoms or until 1/2 of the boiled egg white was ingested. Clinical manifestations of egg allergy for 16 of the 19 patients resulted in skin reactions, which included urticaria (11/19), erythema (5/19), angioedema (2/19), and pruritus (1/19). Several patients had gastrointestinal (4/19) and respiratory (3/19) symptoms. Systemic anaphylaxis occurred in only one patient (SH). Some patients were also sensitive to other allergens such as those found in milk (58% of patients), soybean (25%), wheat (37%), and mites (21%) (unpublished data).

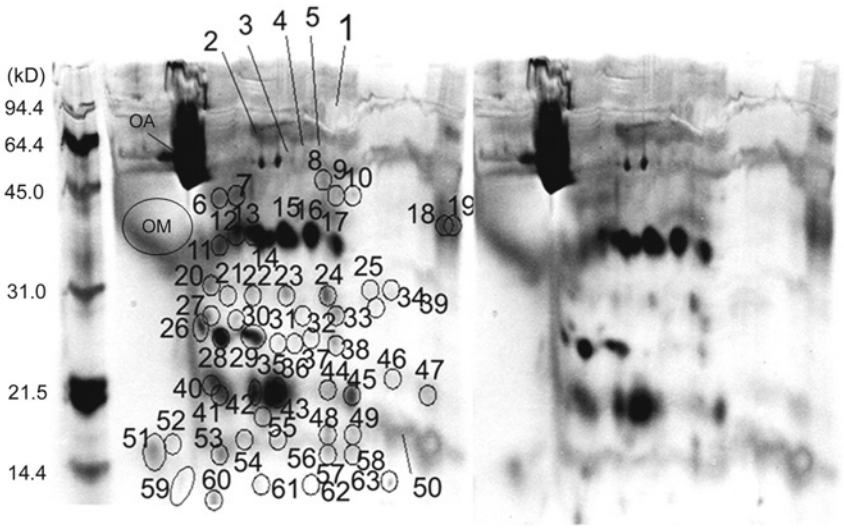

Fig. 1 2-D proteomics map for egg white proteins. Egg white protein was separated by 2-D electrophoresis. Silver staining was performed to visualize total protein. Molecular weight standards (MW) are shown on the left. OA, ovalbumin; OM, ovomucoid.

2-D GEL ELECTROPHORESIS

2-D gel electrophoresis was performed using modified protocols based on Görg *et al.*[10] Egg white from white leghorns was stirred for 40 minutes in 7 M urea, 2 M thiourea, 1% NP-40, and 10 mM Tris-HCl. Protein concentration was determined using a Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA), and egg white was diluted in lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM DTT, and 0.001% bromophenol blue. Diluted egg white was applied to 7-cm immobilized pH3-10 nonlinear gradient strips (Amersham Bioscience, Uppsala, Sweden). First-dimensional isoelectric focusing was performed at 20℃ for 16856 volt-hours using an Ettan IPGphor Isoelectric Focusing System (Amersham Bioscience). After focusing, the gel strips were equilibrated as described by Fujimura *et al.*,[15] although time of each equilibration step was prolonged to 15 minutes. Proteins were subjected to SDS-PAGE as described by Laemmli.[21] Strips were placed on top of 2-D 12% acrylamide gels (10 × 10 × 0.1 cm slab). After migration, gels were fixed and stained in 0.25% Coomasie blue R250, 50% methanol, and 5% acetic acid or with a SilverQuest Silver Staining Kit (Invitrogen Life Technologies, Carlsbad, CA, USA). The protein spots for the study were cut out from acrylamide gel with a clean scalpel.

PROBING IMMUNOLABELING WITH SERUM FROM ALLERGEN-SENSITIVE PATIENTS AND CONTROLS

For the detection of IgE bound to separated egg white proteins, immunolabeling was performed on the patient sera sample from each of the 19 individuals with egg allergy and 11 control individuals. After 2-D PAGE, separated proteins were electroblotted onto PVDF membrane (Amersham Bioscience UK Ltd, Buckinghamshire, England) using a semidry electrophoretic apparatus for 30 minutes at 15 V. The membranes were blocked with 3% (w/v) BSA in 50 mM Tris-HCl (pH 7.4) and 150 mM NaCl. After blocking overnight at 4℃, patient and control sera were diluted 4 times and were added to a washing solution (0.1% Tween 20, 1% BSA) prior to being incubated for 3 days at 4℃. After washing, the membranes were incubated for 3 hours at room temperature with peroxidase-conjugated antibodies consisting of goat antihuman IgE (KPL, Gaithersburg, MD, USA) that had been diluted 1 : 2000 in washing solution. Protein spots resulting from immunolabeling were visualized by reaction with an ECL-plus western blotting detection reagent (Amersham Biosciences) on X-ray film.

TRYPSIN IN-GEL DIGESTION OF PROTEINS ON 2-D GEL

In-gel digestion was performed using modified protocols.[21,22] We collected the same protein spots from multi gels as required. For LC-MS/MS, Coomasie blue-stained spots of interest were excised from 2-D gels (small amounts of spots were collected from multi gels) and incubated 3 to 5 times with 50 mM ammonium carbonate in methanol for destaining. The gel pieces were digested in a buffer containing 100 mM ammonium bicarbonate and 5 ng/μl of modified sequencing grade trypsin (Promega, Madison,

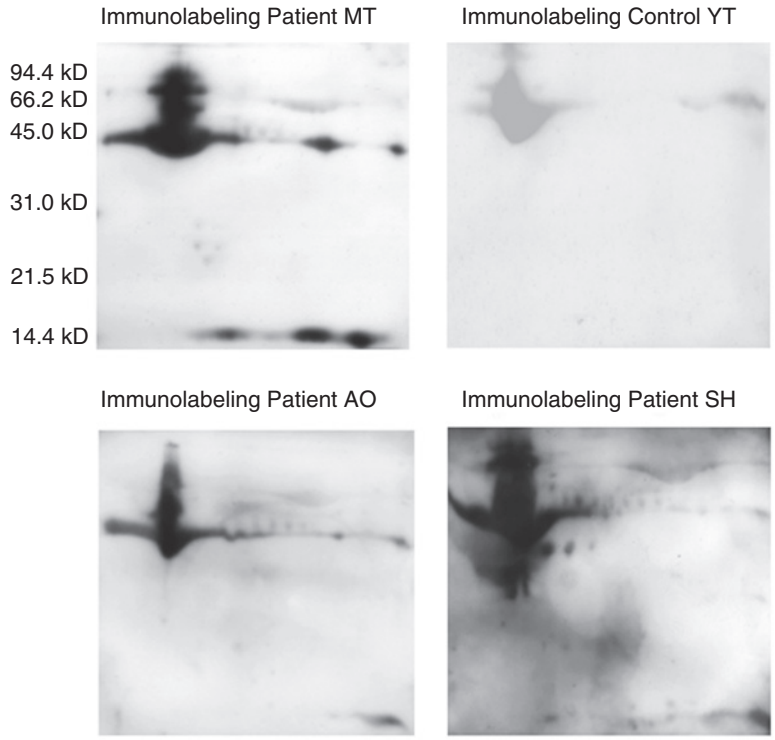

Fig. 2 Immunolabeling with sera from patients MT, AO, SH, and control serum. Molecular weight standards (MW) are shown on the left.

WI, USA). After digestion, the resultant peptides were extracted 3 times each, by sonication for 20 minutes. The extraction was carried out in buffer containing trifluoroacetic acid (TFA, 0.1%) and acetonitrile (5%, 50%, and 95%). Finally, solutions were collected and dehydrated in a centrifugal vaporizer. Proteins were digested in the same way for MALDI-TOF/MS. After digestion, peptide extraction was carried out using sonication in 50% acetonitrile and 1% formic acid. Sonication was performed twice for 10 minutes at each replication and the resulting supernatant was recovered. Collected solutions were concentrated and immediately used for MALDI-TOF/MS.

LC-MS/MS ANALYSIS

Each sample of peptides that had dried after in-gel digestion was reconstituted in a reverse phase buffer A containing acetonitrile/water/formic acid (5 : 95 : 0.1, v/v/v) and subsequently transferred to a Shimadzu auto-sampler (Shimadzu Corporation, Kyoto, Japan). The LC-VP series HPLC system and MS analysis as described previously was used for high-performance LC-MS.[23,24] Triple play scanning sequence data dependent mode was used for Ion trap mass spectrometer. The process from two-dimensional electrophoresis to MS was repeated and confirmed 3 times for newly identified proteins. Raw data were converted to a specific data format, and data analysis was conducted to identify protein performed automatically using Mascot sequence database-searching software (Matrix Science, London, UK). All procedures conducted for the 2D-electrophoresis and the LC-MS/MS analysis were repeated 3 times, and the reproducibility was confirmed.

MALDI-TOF/MS ANALYSIS

The MALDI-TOF/MS analysis method used here was modified as described previously.[22,25] The digests were dissolved in 0.1% TFA and saturated α-cyano-4-hydroxycinnamic acid solution (in 50% acetone, 0.03% TFA) was used as the matrix. The matrix solution was spotted onto an Anchortip (Bruker-Franzen Analytic, Bremen, Germany), and the analyte solution was deposited. TFA (0.1%) was added onto these depositions and then removed immediately. The α-cyano-4-hydroxycinnamic acid solution (0.1 mg/ml) dissolved in 60% EtOH, 30% acetone, and 0.01% TFA was used for recrystallization. Data acquisition and analysis was performed using flex control and flex analysis/biotools version 2.2 software, respectively.

RT-PCR FOR LIPOCALIN-TYPE PROSTAGLANDIN D SYNTHASE (L-PGDS) EXPRESSION

Total RNA was extracted from the magnum of white leghorn using the Isogen RNA Isolation kit (Nippon Gene, Tokyo, Japan). Total RNA (5 μg) was used for the synthesis of first-strand cDNA (called L-PGDS).

Table 2 Reactivities of 2-D spots to sera IgE. 2-D spots immunolabeled by sera from 19 patients and 11 controls

		Spot No.																								
		1*	2	3*	4*	5*	6	7	12	17	18	19	24	25	27	28	29	30	33	37	46	47	48	49	50*	56
	SH	+		+	+	+	+	+								+	+						+	+	+	
	SK	+	+	+	+	+	+	+															+	+	+	
	HS	+														+	+						+	+	+	
p	AO	+	+	+	+	+						+				+	+				+	+	+	+	+	
a	RS	+												+							+	+	+		+	
t	TE	+		+	+	+							+													+
i	AK						+																+	+	+	
e	KM																						+	+		
n	RH		+	+	+	+							+	+											+	
t	KK	+	+	+	+	+								+		+				+			+	+		
	AS																						+	+		
	YY																									
	MY	+					+																+			
	KS	+					+	+	+															+		
	MT	+													+	+		+							+	
	HM	+																	+				+		+	
	KS	+		+	+	+																			+	
	SS	+	+	+	+	+	+	+		+			+											+	+	
	RY	+		+	+	+	+				+												+	+	+	
	KH																									
c	TH						+	+																		
o	SY																						+	+		
n	MK		+																				+	+		
t	KM																		+	+						
r	TW																									
o	YT																									
l	AT																									
	TM																									
	HM																									
	RH										+	+							+				+	+	+	
P	%	74	21	53	53	53	32	21	5	5	5	5	16	16	5	26	16	5	5	5	16	16	63	53	53	5
C	%	0	9	0	0	0	9	9	0	0	9	9	0	0	0	0	0	0	18	9	0	0	27	27	9	0

Asterisks indicate significant differences between egg allergy patients and controls by positive percentage.

The resulting cDNA was stored at -20℃ until use. The oligonucleotide primer was synthesized by using the following DNA sequences applied restriction enzyme sites (EcoRI, SalI): EcoRI-PGDS primer: 5'-C CGAATTCCATGCAAGCCACGCTGCTCSGC-3'; SalI-PGDS primer: 5'-CCCCGTCGACCTAGGCAGCATCT GCCATAC-3'.

PCR was performed in a 50 µl final reaction volume containing 1 µl cDNA, 0.5 µM of each sense and antisense primers, 0.2 mM of each dNTP, PCR 1 × buffer, 1.25 U of Pyrobest DNA polymerase (Takara, Shiga, Japan). The resulting PCR product was cloned into the pGEX6P-3 vector (Amersham, Piscataway, NJ, USA). Cloned L-PGDS was sequenced by an ABI Prism 310NT Genetic Analyzer.

PURIFICATION OF RECOMBINANT L-PGDS

Cloned L-PGDS was expressed as a glutathione S-transferase fusion protein in BL21 E.coli. The recombinant fusion protein was purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B (Amersham, Uppsala, Sweden) and the glutathione S-transferase fusion tag was removed by PreScission Protease cleavage (Amersham, Piscataway, NJ, USA).

IMMUNOLABELING OF CYSTATIN AND RECOMBINANT L-PGDS WITH SERA FROM PATIENTS AND CONTROLS

Cystatin (SIGMA, St. Louis, MO, USA) and recombinant L-PGDS were separated by SDS-PAGE using a 13% or 12% separation gel. Five hundred nanograms (ng) of cystatin or 1 µg of recombinant L-PGDS was applied to each well. Each electroblotted membrane was stained with Ponceau S or CBB and then immunolabeled as described above.

Table 3 Identification of egg white protein using LC-MS/MS and MALDI-TOF/MS analysis

Spot	Protein Description	Accession No.	Determined Sequence	Method†
1	Ovotransferrin	gil229552	SDFHLFGPPGKK	MALDI-TOF/MS
			KDSNVNWNNLK	
			IQWCAVGKDEK	
			DLLFKDSAILMK	
			KGTEFTVNDLQGK	
			SAGWNIPIGTLLHR	
			FFSASCVPGATIEQK	
			TDERPASYFAVAVAR	
			NAPYSGYSGAFHCLK	
			DDNKVEDIWSFLSK	
			GAIEWEGIESGSVEQAVAK	
			ECNLAEVPTHAVVVRPEK	
			TGTCNFDEYFSEGCAPGSPPNSR	
			WSVVSNGDVECTVVDETKDCIIK	
			HTTVNENAPDQKDEYELLCLDGSR	
			LKPIAAEVYEHTEGSTTSYYAVAVVKK	
			IMKGEADAVALDGGLVYTAGVCGLVPVMAER	
28	Ch21 protein	gil20178282	EVSPTAMAIFR	LC-MS/MS
			ERNYTDEMVAVLPSQEECSVDEV	
			ISFLGEDELEVSYAAPSPK	
29	Lipocalin-type prostaglandin D synthase	gil45383612	STGSSNMVLLYSR	LC-MS/MS
			MCTTDIAVTADGNMEVTSTYPK	
43	HEP21 protein	gil45383131	VTLYYQQGCTSALMCGRER	LC-MS/MS
			TSLGKVTLYYQQGCTSALNCGR	
45	HEP21 protein	gil45383131	VTLYYQQGCTSALMCGRER	LC-MS/MS
			TSLGKVTLYYQQGCTSALNCGR	
			VATVSLPR	
			YSCCETDLCNEK	
49	Cystatin	gil118195	ALQFAMAEYNR	LC-MS/MS
			LLGAPVPVDENDEGLQR	
50	Lysozyme mutant with Trp 62 replaced by Tyr	gil1065027	FESNFNTQATNR	MALDI-TOF/MS
			IVSDGNDMNAWVAWR	
			NTDGSTDYGILQINSR	
			NLCNIPCSALLSSDITASVNCAK	
			NTDGSTDYGILQINSRYWCNDGR	

† Protein spots analyzed and identified using either LC-MS/MS or MALDI-TOF/MS.

RESULTS

ELECTROPHORETIC PROFILES OF EGG WHITE ALLERGENS

In preliminary studies, the number of egg white proteins on pH3-10 non-linear 2-D gels was greater than that of pH4-7 2-D gels; routine analysis was thus performed on the former. In 2-D gel stained by CBB, the number of protein spots was 30 (unpublished data), whereas 63 were observed on silver-stained 2-D gels (Fig. 1).

Protein spots were immunolabeled with serum IgE from both patients with egg allergy and control subjects. Representative immunoblot results are shown in Figure 2. Twenty-five of the 63 spots observed by silver staining reacted with patient sera. Fifteen of these spots (nos. 1, 3, 4, 5, 12, 17, 24, 25, 27, 28, 29, 30, 46, 47, and 56) specifically reacted with patients' sera, although spots no. 12, 17, 27, 30, and 56 showed IgE-reaction only to a single serum (Table 2). Ten spots (nos. 2, 6, 7, 18, 19, 33, 37, 48, 49, and 50) reacted with both control and patients' serum IgE. Although spot no. 50 was detected in more than half of the patients, only one control subject was positive. The unknown 38 spots (nos. 8-11, 13-16, 20-23, 26, 31, 32, 34-36, 38-45, 51-55, 57-63) did not react with either control or patient serum IgE.

IDENTIFICATION OF IgE-REACTIVE SPOTS

To identify IgE-reactivity, spots were excised, di-

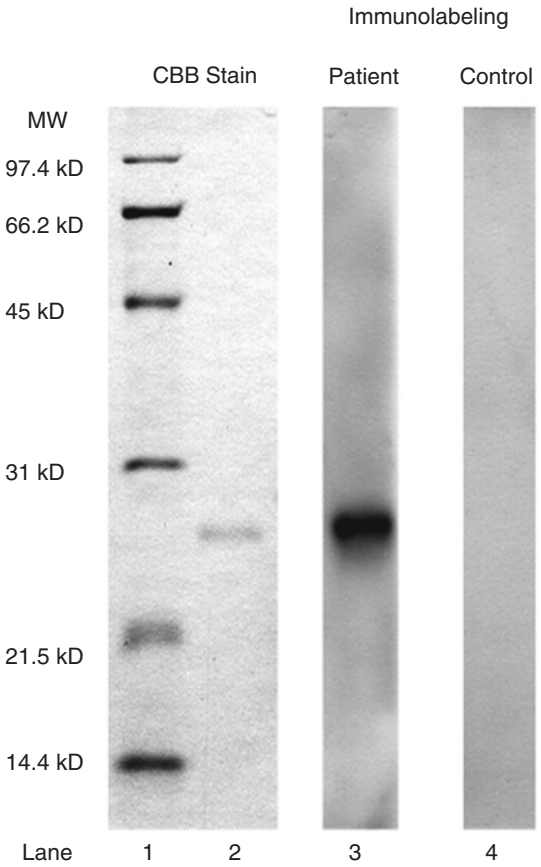

Fig. 3 Immunolabeling of recombinant L-PGDS with sera from patients and controls. Molecular weight standards (MW) are shown on the left. Patient, SH; Control, TM.

gested in-gel with trypsin, and the resulting peptide mixtures were analyzed by LC-MS/MS or MALDI-TOF/MS. Table 3 shows the peptides identified for each protein.

Spot no. 1 reacted with 74% and spot no. 50 reacted with 53% of the egg allergy patients; confirmed as ovotransferrin and lysozyme, respectively. These results have been previously reported.[6] Moreover, we could confirm that reactivity to Ovomucoid (OM) in this study corresponded to the OM-specific ImmunoCAP (unpublished data). Spots no. 43 and 45, which had no reactivity to IgE, were both identified as HEP21. We showed for the first time that spots no. 28 and 29, also known as ch21 and lipocalin-type prostaglandin D synthase (L-PGDS), respectively, reacted with IgE from patients with egg allergy. We also show that spot no. 29 reacted with 16% of patients with egg allergy (including one patient with systemic anaphylaxis). Finally, spot no. 49 showed as high reactivity as lysozyme mutant activity with Trp 62 being replaced by Tyr in the sera of 53% of the patients, which was identified as cystatin. Patient YY did not react to any spots other than OA.

IMMUNOLABELING OF RECOMBINANT L-PGDS AND PURIFIED CYSTATIN

We confirmed whether recombinant L-PGDS and cystatin of allergen candidates in this study react with serum from egg allergy patients by purification of egg white proteins. Figure 3 and 4 show that L-PGDS and cystatin bind to IgE from egg allergy patients.

DISCUSSION

We set out to determine whether novel egg white proteins reacted with IgE in patients with egg allergy and identified the proteins by conducting 2-D immunolabeling and mass spectrometric analysis. Recently, new allergens have been demonstrated from sesame seeds, Japanese cedar pollen, shrimp, etc., using 2-D immunolabeling in combination with MALDI-TOF/MS.[15-18] These reports have found that the combination of 2-D immunolabeling and mass analysis effectively identifies new allergens. We were able to perform this study using a proteomic method and the LC-MS/MS system.[23] The LC-MS/MS system used in the present study has already been verified by us in an earlier experimental report.[24] Although Guérin-Dubiard *et al*.[5] reported the identification of 67 spots in egg white by multiple immobilized pH gradient 2D gels, we were able to reveal 63 spots using one gel (pH3-10NL 2-D gel) and silver staining. In examinations, it is important that multiple proteins can be analyzed using one gel.

2-D-immunoblot results indicated that many proteins in egg white react with IgE in serum. The 25 spots that reacted with patients' sera could be allergen candidates. We demonstrated that spots no. 1 (ovotransferrin) and 50 (lysozyme) reacted more intensively with serum from patients with egg allergy compared to controls; and these 2 have already been reported as major allergens.[6] We also revealed, for the first time, IgE-reactivity to ch21 protein, L-PGDS, and egg white cystatin on 2-D gel. Reaction to L-PGDS and cystatin was confirmed using each purified protein.

Ch21 protein and L-PGDS belong to the lipocalin family,[26] which has been reported as allergenic.[27,28] Ch21 protein and L-PGDS were minor allergens. However, the sera from patient MT, which reacted to spot no. 28 did not seem to react to the major spots, such as nos. 3, 4, 5, 48, or 49. Patient HS did not react to nos. 3, 4, 5 spots, either. This may increase the possibility that the newly identified allergens are not only the hidden allergen components, but there are some patients who are preferentially reacting to those minor egg allergens. Previous reports show that being a minor allergen does not always mean that it is allergenically poor.[29,30] Tomato profilin Lyc e 1, which is a minor allergen, induces histamine release.[29] These reports suggest that minor allergens are targets for desensitizing treatments. Urade *et al*. reported that PGD_2 synthesized by L-PGDS is related

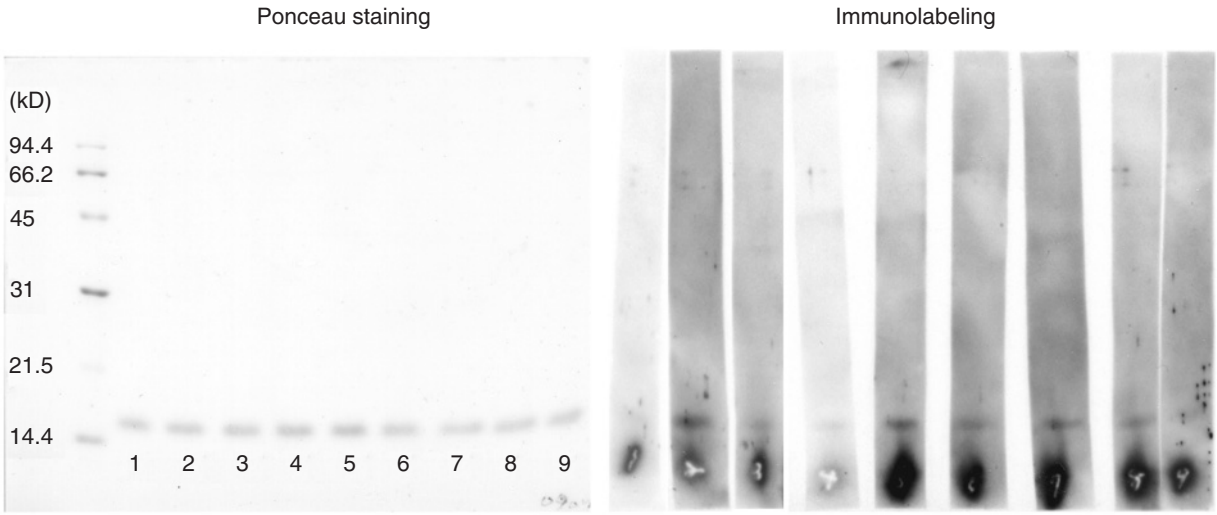

Fig. 4 Immunolabeling of cystatin with sera from patients and controls. Molecular weight standards (MW) are shown on the left. Lane 1, control YT; lane 2, patient SS; lane 3, patient KM; lane 4, patient KK; lane 5, patient KS; lane 6, patient AK; lane 7, patient AO; lane 8, AS; lane 9, patient SH.

to allergic inflammation in vivo and that L-PGDS from chicken shows PGDS activity.[31,32] These reports suggest that L-PGDS induces allergic reactions in addition to binding to IgE, and that PGD_2 may exist in eggs.

Cystatin has been reported to be a cysteine protease inhibitor, and is an allergen in cats and dogs.[33-35] Cystatin was considered to be a major allergen because it showed high reactivity to IgE from patients with egg allergy. There are two possible reasons for the lack of literature on cystatin as an allergen. First, the similarity in molecular weight of cystatin and Lysozyme, a major allergen, makes it difficult to identify cystatin without using 2-D gel electrophoresis. Further, the presence of only a small amount of cystatin in eggs makes it difficult to identify without development of LC-MS/MS. Guérin-Dubiard *et al*.[5] identified a spot as cystatin for the first time in 2006. We demonstrated the reactivity of cystatin with IgE from egg allergy patients using these developments in technology.

IgE-reactivity profiles, including undefined proteins in egg white, are shown in Table 2. The data and methods here will provide guidance in determining the sensitivity of individual patients to certain protein or combination of proteins. Such information will be useful for making more accurate diagnoses and patient-tailored desensitizing treatments. Many studies on egg allergens have been performed using purified egg allergens such as ovalbumin and ovomucoid. However, some allergens may interact with each other, other proteins or other components in eggs. This may in turn relate to the degree of allergic inflammation.

In conclusion, we demonstrated for the first time, using proteomic analyses, some novel egg white proteins that bound to IgE. We also identified new allergen candidates. Our proteomics-based analyses in egg white will support the development of more accurate diagnoses and patient-tailored therapies for egg allergy sufferers.

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